REMARKS

The present amendment is in response to the Office Action in the above-identified application mailed on August 1, 2001. Claims 1-21 are pending. Claims 3, 7, 14 and 15 have been cancelled. Claims 1, 4, 5, 8, 9, 11, 12, 16 and 17 have been amended. The Specification and Drawings have also been amended. Claims 18-21 have been added. A marked-up version of the new and amended claims is supplied at the end of this response as required under 37 C.F.R. 1.121(c)(1).

All amendments and added claims are supported by the specification, drawings and claims as filed. No new matter is added by this amendment.

Sequence Compliance

The Examiner has requested that a sequence listing be filed for the GAL4 sequences referenced in Figure 1, or, alternatively, that the designation "GAL4(1-147)" be replaced with "GAL4BD" and that the designation "GAL4(768-881)" be replaced with "GAL4AD" in Figure 1 and throughout the specification.

Accordingly, Applicants have amended Figure 1 to make the suggested replacements. Applicants have additionally amended the specification so that the descriptions of Figures 2 and 3 in the Brief Description of the Drawings omit the reference to portions of the sequence. Applicants agree with the Examiner that one skilled in the art will understand which residues are encompassed by the GAL4 binding domain and the GAL4 activation domain.

Therefore, Applicants submit that no sequence listing is necessary for the present application and request that the Examiner's objection be withdrawn.

Drawings

The Examiner has indicated that Figure 1 should be designated as "Prior Art". Accordingly, Applicants have made such amendment to the figure.

The Examiner has also objected to Figures 4 and 5 as presenting a unit (10^{-n} M), which the Examiner finds unclear. Applicants submit that one skilled in the art would readily determine that n represents the number on the x-axis. This is true for several reasons.

First, it is common graphical notation to include a variable that represents any given unit on an axis in the name of that axis. So long as there is only one variable, as in this case, no confusion should result.

Second, given that "n" is the exponent of 10 and the x-axis is clearly depicting exponential intervals, one would conclude that "n" must be the number designated on the axis.

Third, one would not conclude, as the Examiner suggests, that the designation means something else, *e.g.* 15 X 10^{-n}. Such a reading would insert a variable before the 10^{-n} designation that simply is not there. One skilled in the art would have no reason to and would not insert a variable where one does not exist and thereby leave an existing variable undefined.

However, because clarification may easily be made and may allow one skilled in the art to more immediately grasp the meaning of the figures, a sentence has been inserted in the Brief Description of the Drawing for each of Figures 4 and 5. Such insertion is made solely to advance prosecution of the application and to make it accessible even to those less than skilled in the art and should not be construed as an admission that any defect or lack of clarity exists in the drawings as filed.

Additionally, Applicants submit that one skilled in the art would not be confused by the fact that numbers decrease from left to right on the x-axis because one skilled in the art would readily comprehend that, because the numbers represent the negative exponent of 10, the graph actually depicts an *increasing* dose from left to right. Applicants note that this provides further evidence as to why one skilled in the art would understand that "n" is the number on the x-axis.

Additionally, a typographical error in Figure 3 in which "GAL4pBD" was designated at "GAL4DBD" has been corrected.

Accordingly, Applicants believe that all drawings are now correct, clear and bearing proper designations. Withdrawal of all objections to the drawings is respectfully requested.

Specification

The Examiner has objected to various informalities in the Specification. First the Examiner has objected to the reversal of the descriptions for Figures 2 and 3 in the Brief Description of the Drawing and the Detailed Description of the Preferred Embodiments.

Applicants believe that figures are identified in a partially correct manner on pages 7 and 8, in that the first half of the paragraphs describing Figures 2 and 3 are correct while the second, figure legend halves are incorrect. Other inconsistencies have been noted and Applicants have according amended the Specification to correctly reference these figures. Applicants submit that one skilled in the art would not have been confused by these errors in the application as filed because the abbreviations are defined correctly in the application, albeit in the wrong paragraphs and because it is clear that Figure 2 comprises the activation domain while Figure 3 comprises the binding domain.

The Examiner has also objected to Table 1, at page 19 for failing to provide units. Applicants submit expression from the *lac Z* reporter gene in two-hybrid assays is frequently not designated with any unit. This is because those skilled in the art understand that *lac Z* expression is traditionally measured in Miller units, which are an arbitrary unit. See *e.g.* the three Guarente et al. references from Applicants' Information Disclosure Statement. However, to avoid any confusion Applicants have amended the table to indicate that units are Miller units.

Finally, the Examiner has objected to the use of the terms "Gal4pBD DNA binding domain" and "Gal4pAD transcriptional activation domain" as used in Claim 17. Although Applicants allege that the meaning of these terms is clear from page 7, line 20 and page 8, line 2, respectively, Applicants have nevertheless amended the Specification and Claim 17 so that its language matches that of the Specification exactly.

In light of the above, Applicants believe the Specification is clear and in appropriate form and request withdrawal of all objections.

Claim Objections

The Examiner has objected to Claims 7, 11, 12 and 17 because of several informalities. In response to the Examiner's objections, Claim 7 has been cancelled and Claims 11 and 12 have been amended so that "mineralocorticoid" is listed only once. In Claim 17, "inducible" has been spelled correctly, an "in" has been changed to "with", the problems with "Gal4pBD" and "Gal4pAD" have been corrected, and "resistance genes" has been made singular.

In light of these changes, Applicants submit that the claims are fee of minor defects and request that the Examiner withdraw all claim objections.

Rejections under 35 U.S.C. §112, ¶1, Enablement

The Examiner has rejected Claim 1-16 under 35 U.S.C. §112, ¶1 as not enabled for interacting molecules other than proteins. Applicants have accordingly limited the scope of these claims to proteins. Applicants reserve the right to pursue additional interacting molecules in continuation or continuation-in-part applications. Additional changes to specify the nature of the hybrid proteins have also been made. Claim 3 and 7 have been cancelled.

Therefore, all claims are now adequately enabled and withdrawal of the rejection under §112, ¶1 for lack of enablement is respectfully requested.

Rejections under 35 U.S.C. §112, ¶1, Written Description

The Examiner has rejected Claims 12-16 under 35 U.S.C. §112, ¶1 for failure of the written description requirement. Specifically, with regard to Claim 12, the Examiner appears to have has alleged that Applicants have failed to demonstrate possession of small molecules as interacting agents. Alternatively, it appears that the Examiner may have alleged that Applicants failed to demonstrate possession of small molecules as modulatory agents for levels of interacting molecules within the host cell. Applicants are not certain which allegation is the basis of the rejection.

Regardless of the basis for the rejection, Applicants submit that Claims 12-16, as amended, adequately demonstrate possession of the invention as claimed at the time of filing. Applicants no longer claim small molecules as interacting molecules, so any rejection premised on this basis is now moot.

Applicants further submit that, when read correctly, Claim 12 claims the use of small molecules as modulatory agents for levels of the first and/or second hybrid proteins. Applicants include a large number of small molecules in Claim 12 and in new dependent Claims 18-21. Additional small molecules suitable for use as modulatory agents would be apparent to one skilled in the art. Essentially, any small molecule capable of causing a change in expression of the first or second hybrid protein would be suitable. Thus, appropriate small molecules for any given two-hybrid system will vary depending upon the exogenously activatable promoter chosen to drive expression of a hybrid protein. The small molecule might act directly on the promoter,

or it might act at any point during a signal transduction pathway to cause gene expression via the promoter.

Systems and genes activated by such molecules, such as the steroid response pathway, are currently well-documented and more are being discovered constantly. Thus, one skilled in the art would readily be able to peruse the literature and determine which cellular pathways and small molecules are appropriate for a given two-hybrid experiment. Ideally, choices should be made to avoid interference with the bait/prey interaction, to avoid pathways commonly used by the host cell, and to avoid death of the host cell. This also would be apparent to one skilled in the art.

Applicants accordingly submit that possession of the subject matter of every claim at the time of filing has been adequately demonstrated. Withdrawal of the §112, ¶1, written description rejection is requested.

Rejections under 35 U.S.C. §112, ¶2

Claims 1-17 have been rejected under 35 U.S.C. §112, ¶2 as indefinite. Claim 1 has been amended so that the preamble better relates to the steps of the claim and to indicate that amounts of both hybrid proteins may be regulated.

The Examiner has indicated that he believes the use of the terms "capable of" and "capacity" denotes only a latent ability which may or may not be activated and therefore renders the claim indefinite. Applicants disagree. First, the use of such terms is necessary to accurately

portray the invention. The DNA binding region is merely capable of binding DNA. There is no guarantee that every hybrid protein comprising such a region is bound to a DNA molecule. In fact, some possible negative and positive effects of this are discussed in the Specification and form the basis for creating an adjustable system. Similarly, there is no guarantee that each transcriptional activator will actually activate transcription. This first requires an interaction between the bait and prey protein (or a few accidental activations). The implications of this and its effects on an adjustable system are also discussed in the Specification. Finally, there exists only a capacity to regulate the absolute or relative amounts of the hybrid proteins because in some systems this latent capacity will not be used.

It will be readily apparent to one skilled in the art when, for instance, the DNA binding domain is more likely to actually bind DNA, *i.e.* there are equal or lower numbers of the first hybrid protein than DNA binding sites. One skilled in the art will also understand that it is highly unlikely that all DNA binding domains will actually be bound to DNA. Similarly, one skilled in the art will known when the capacity to regulate transcription and thus levels of the two hybrid proteins should be used, as indicated in the Specification, page 12, line 10 through page 13, line 3, to adjust the sensitivity of the assay system.

Furthermore, a number of recently issued patents use "capable of" language in similar situations. See:

- U.S. Pat. No. 6,310,271, Claim 1, "polynucleotide molecule being *capable of* hybridizing to a polynucleotide",

- U.S. Pat. No. 6,310,270, Claim 27, "providing a targeting vector *capable of* disrupting said eNOS gene upon homologous recombination",
- U.S. Pat. No. 6,310,044, Claim 1, "contains a sequence CAT and is *capable of* inhibiting activity of a herpesvirus",
- U.S. Pat. No. 6,310,035, Claim 2, "said nucleotide sequence being *capable of* hybridizing with a gene",
- U.S. Pat. No. 6,310,034, Claim 25, "said immunogen being *capable of* eliciting an immune response",
- U.S. Pat. No. 6,309,868, Claim 13 "Aspergillus oryzae *capable of* providing at least 50 mU of prolyl-dipeptidyl-peptidase activity per ml of supernatant", and
- U.S. Pat. No. 6,309,868, Claim 1, "a protein *capable of* forming a potassium (K.sup.+) ion channel".

Hundreds of similar examples exist, but are not listed here for brevity's sake. Applicants fail to understand why the use of "capable of" is acceptable in the above examples and other issued biotech patents and not in Applicants' claims. Should the Examiner persist in his rejection based upon use of this language, clarification is respectfully requested.

Claim 3 has been cancelled, rendering the rejection of this claim moot.

The Examiner has rejected Claim 4 for containing the language "capable of". For the reasons explained above in relation to Claim 1, Applicants do not believe that this is indefinite and request further clarification.

Claim 7 has been cancelled, rendering the rejection of this claim moot.

Claim 9 has been clarified as requested.

Claim 11 has been amended to recite a proper Markush group and to further clarify the claim as requested by the Examiner. Retinoid is not listed as a steroid in this claim, but rather as an exogenous activator.

Claim 12 has been amended to remove potentially confusing examples of the claimed types of molecules. These examples have been moved to four dependent claims, Claims 18-21, which recite a proper Markush group and further clarify the claimed material as requested by Examiner. "Continuous" adjustment of hybrid protein levels and reporter sensitivity is discussed in the Specification, *inter alia*, at page 11, line 19 through page 13, line 3 and at page 14, line 21 through page 15, line 3. Thus this term is defined in the specification. Reference to "discontinuous" adjustment has been cancelled. Applicants reserve the right to pursue claims to discontinuous adjustment in continuation or continuation-in-part applications.

Similarly, the term "continously adjustable" as used in Claim 13 is defined in the Specification, *inter alia*, at page 11, line 19 through page 13, line 3 and at page 14, line 21 through page 15, line 3. The term "plurally stepped dose-responsive basis" is discussed in the Specification, *inter alia*, at page 14, line 21 through page 15, line 3 and in Examples 2 and 3.

Claims 14 and 15 have been cancelled, rendering the Examiner's rejections of these claims moot.

The Examiner has rejected Claim 16 for recitation of the claim language "capable of". Applicants disagree that this language is indefinite for the reasons previously set forth with respect to Claim 1.

Claim 17 has been amended to introduce various clarifications as recommended by the Examiner. Applicants again assert that use of the language "capable of" is no inappropriate in this claim for the same reasons as set forth in Claim 1.

Applicants therefore submit that all remaining pending claims are definite and particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Withdrawal of the rejection under §112, ¶2 is requested.

Rejections under 35 U.S.C. §102(b)

The Examiner has rejected Claims 1-9 under 35 U.S.C. §102(b) as anticipated by Finley et al. Applicants submit that Claim 1, as amended, is not anticipated by Finley et al. Specifically, Claim 1 has been amended to recite continuously adjustable regulation of the amounts of the hybrid proteins. Finley et al. provide for regulation only between an on/induced and off/basal/uninduced level of expression in the cell. Such as system is far from continuously adjustable. Thus the reference provides little guidance for the system of the present invention, which allows a variety of levels of expression. For further explanation of the kinetics of the

present system, which should make the difference between Applicants' system and that of Finley et al. more apparent, please see the attached reference from Applicants' Declaration Under 37 C.F.R. 1.132 of David Edwards.

Withdrawal of the rejection under §102(b) is respectfully requested.

Rejections under 35 U.S.C. §103(a)

The Examiner has rejected Claims 1-15 under 35 U.S.C. §103(a) as obvious in light of Finley et al. as applied to Claims 1-9 under §102(b) and in view of Schena et al. As explained above, Finley et al. does not disclose a method for continuously adjustable regulation of hybrid protein levels. Further, in its simple description of an on/off interaction detection system, it fails to even suggest, much less provide any guidance as to how a continuously adjustable system might be achieved. Accordingly, Claims 1-9 are not obvious in light of Finley et al. As indicated by the Examiner, Claims 10-15 are not obvious in light of Finley et al. because it fails to address the use of a system of inducible promoters based on steroid receptors. The difference between Applicants' invention and that of Finley et al. should become more apparent through reference to Applicants' Declaration Under 37 C.F.R. 1.132 of David Edwards.

Furthermore, none of Claims 1-15 are rendered obvious when the disclosure of Schena et al. is added to that of Finley et al. Applicants first note that there is no reason to combine the two references unless the Examiner believes that it would be obvious to combine any inducible promoter system with the yeast two-hybrid system. Should that be the case, the Examiner, by the same logic, should agree with the arguments presented above in relation to written

description that one skilled in the art would understand that any inducible promoter system with appropriate exogenous activators may be used with the present invention.

However, the claims as amended are not obvious in light of Finley et al. and Schena et al. because, as the Examiner recognizes, Schena et al. primarily teaches the use of steroid hormone-based inducible promoter system. This merely adds a second promoter system that may be used as an on/off switch in the Finley et al. yeast two-hybrid system. It does not in any way suggest or enable the use of a continuously adjustable system for interaction detection.

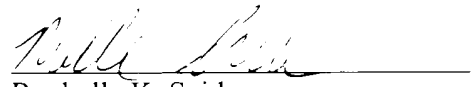
Accordingly, Applicants request that the Examiner withdraw all rejections under §103(a).

CONCLUSION

Applicant believes Claims 1-2, 4-6, 8-13 and 16-21 are in condition for allowance. A Notice of Allowance is respectfully solicited. Applicants believe that a fee of $200 is required for a one-month extension of time under 37 C.F.R. 1.17(a)(2). Accordingly, a check in that amount is enclosed. Should any additional fees be due as a result of this amendment or for any other reason during prosecution of this application, the Commissioner is hereby authorized to charge the payment of any required fees to Deposit Account No. 02-4377.

Respectfully submitted,

BAKER BOTTS, L.L.P.



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please replace the paragraph beginning at page 7, line 13 and ending at page 7, line 20 with the following amended paragraph:

Figure 2 shows the Novel Library vector described herein is a shuttle vector containing ampicillin and the colE1 origin of replication for selection in E.coli as well as TRP1 and the 2 micron origin of replication for selection in yeast. Unknown cDNAs are fused to the Gal4p activation domain, and continuously variable expression is obtained by the induction of GRE upstream activating element(s) attached to CYC1 promoter. [KAN= Kanamycin] AMP = Ampicillin, *E.coli* selectable marker; ori= colE1 bacterial origin of replication; [URA= URA3 gene, yeast selectable marker;] TRP= TRP1 gene, yeast selectable marker; 2um= origin of replication for yeast; [ERE= Estrogen Response Element;] GRE= Glucocortocoid Response Element; CYC1p= CYC1 promoter from yeast; [Gal4pBD= Gal4p DNA binding domain (1-147);] Gal4AD= Gal4 activation domain; AdhT= Alcohol dehydrogenase terminator.

Please replace the paragraph beginning at page 7, line 21 and ending at page 8, line 3 with the following amended paragraph:

Figure 3 shows the novel Bait vector in a shuttle vector containing kanamycin and the colE1 origin of replication for selection in E.coli as well as URA3 and the 2um origin of replication for selection in yeast. A known cDNA is fused to the Gal4p DNA binding domain, and continuously variable expression is obtained by the induction of ERE element(s) attached to

CYC1 promoter. [AMP= Ampicillin, *E.coli* selectable marker;] KAN= Kanamycin, *E.coli* selectable marker; ori= colE1 bacterial origin of replication; [TRP= TRP1 gene, yeast selectable marker;] URA= URA3 gene, yeast selectable marker; 2um= origin of replication for yeast; [GRE= Glucocortocoid Response Element;] ERE= Estrogen Response Element; CYC1p= CYC1 promoter from yeast; [Gal4pAD= Gal4 activat. domain (768-881);] Gal4pBD= Gal4p DNA binding domain; AdhT= Alcohol dehydrogenase terminator.

Please replace the paragraph beginning at page 8, line 4 and ending at page 8, line 10 with the following amended paragraph:

Figure 4 shows Continuously Dose Responsive Expression of proteins fused to Gal4pBD in the "Bait Vector" in yHYB001 strain. Strain yHYB001 with the bait vector is grown in selective minimal media with varying concentrations of estradiol. The bait vector contains the Gal4pBD fused to the marker *lacZ*. β-gal expression assays are performed three times per estradiol concentration; the data represents an averaging of three assays per sample. Growth was overnight and strains were at OD_{600} ca. 0.8 when assayed. Strain yHYB001 is described in the text. β-gal expression assays are described in Guarente (1983). The variable "n" in the x-axis label "Dose (10^{-n}) dexamethasone" represents any given number on the x-axis.

Please replace the paragraph beginning at page 8, line 11 and ending at page 8, line 17 with the following amended paragraph:

Figure 5 shows Continuously Dose Responsive Expression of proteins fused to Gal4pAD in the "[Bait] Library Vector" in yHYB001 strain. Strain yHYB001 with the library vector is grown in selective minimal media with varying concentrations of dexamethasone. The [bait]

library vector contains the Gal4pAD fused to the marker *lacZ*. β-gal expression assays are performed three times per dexamethasone concentration; the data represents an averaging of three assays per sample. Growth was overnight and strains were at OD_{600} ca. 0.8 when assayed. Strain yHYB001 is described in the text. β-gal expression assays are described in Guarente (1983). The variable "n" in the x-axis label "Dose (10^{-n}) dexamethasone" represents any given number on the x-axis.

Please replace the paragraph beginning at page 17, line 19 and ending at page 17, line 25 with the following amended paragraph:

In this embodiment, in the first hybrid protein, the bait may be fused to the carboxyl-terminal end of the GAL4bd, a DNA binding domain [(Figure 2)] (Figure 3). This first hybrid protein may be transcribed in a continuous range of amounts over up to five orders of magnitude , and under the influence of an estrogen response element (ERE) within a minimal promoter. This results in variable expression of the bait first hybrid protein over a continuous range of amounts in response to changing levels of estrogen or estrogen antagonists in the yeast growth medium. This promoter-first hybrid protein construct is provided on a two-micron plasmid either under ARG4 or URA3 selection.

Please replace the paragraph beginning at page 18, line 1 and ending at page 18, line 12 with the following amended paragraph:

The second hybrid protein may be formed by fusion of the prey polypeptide, which may be derived from a library, to the carboxyl-terminal end of the GAL4ad , a transcriptional activation domain [(Figure3)] (Figure 2). This second hybrid protein may be transcribed in a

continuous range of amounts over up to five orders of magnitude and under the influence of preferably one to six, and in the present example, three, glucocorticoid response elements (GREs) within a minimal promoter, for example, including but not limited to that from CYC1. This results in variable expression of the second hybrid protein over a continuous range of amounts in response to changing levels of glucocorticoids or their antagonists, including but not limited to dexamethasone, in the yeast growth medium. This promoter- second hybrid protein construct is provided on a two-micron plasmid under TRP1 selection. Both hybrid protein plasmids are also shuttle vectors containing either ampicillin or kanamycin resistance and a colE1 origin of replication, which provide for manipulation in *E. coli* bacteria.

Please replace the paragraph beginning at page 19, line 4 and ending at page 19, line 20 with the following amended paragraph:

As noted above, the Brent lab has shown that a given set of two-hybrid protein interactors yield a uniform quantitative reporter output directly proportional to their strength of interaction (Estojak et al., 1995). Utilizing the novel adjustable yeast interaction hybrid system (IHS), introduced and described as a more preferred embodiment in the paragraphs above, three sets of proteins pairs previously demonstrated to interact in a two-hybrid system are demonstrated to give variable levels of reporter output when expressed at different relative concentrations. The level of expression of the first hybrid protein containing the bait is proportional to the concentration of estradiol, and the level of the second hybrid protein containing the prey derived from a library is proportional to dexamethasone concentration (Kralli et al., 1995; Gaido et al., 1997 (Figures 4 and 5)).

Table 1 - Quantitation of known interactors in a traditional Two-Hybrid Screen (2HS) and the novel Interaction Hybrid System (IHS) at various levels of sensitivity

BAIT HYBRID	LIBRARY HYBRID	TRADITIONAL 2HS	LOW SENSITIVITY IHS	MEDIUM SENSITIVITY IHS	HIGH SENSITIVITY IHS
SNF1	SNF4	300	50	250	2000
Pelle	Tube	250	20	150	1400
Pelle	Dorsal	1300	100	1400	2500

* All quantitations of interactions are in Miller units.

In the Drawing:

Please replace Drawing Sheet 1, Figure 1 and Drawing Sheet 2, Figures 2-4 with the replacements sheets attached hereto.

In the Claims:

Please cancel Claims 3, 7, 14 and 15.

Please amend Claims 1, 4, 5, 8, 9, 11, 12, 16 and 17 as follows:

1.(once amended) A method for detecting an interaction between a first test protein and a second test protein at variable sensitivities via a detectable reporter gene, the method comprising:

(a) providing a host cell [,] wherein the host cell comprises a detectable reporter gene capable of expressing a detectable reporter gene product;

(b) providing to the host cell a first [interacting molecule, which may be a macromolecule or a small molecule,] hybrid protein comprising a polypeptide region capable of binding DNA and a bait polypeptide derived from the first test protein and a second [interacting molecule, which may

be a macromolecule or a small molecule,] hybrid protein comprising a polypeptide region capable of transcriptional activation and a prey polypeptide derived from the second test protein, wherein the host cell is additionally provided with the capacity to regulate the absolute or relative amounts of the first [or] and second [macromolecules or small molecules] hybrid proteins;

(c) regulating the amounts of the first [or] and second [macromolecules or small molecules] hybrid proteins in a continuously adjustable manner so the detectable reporter gene is activated; and

(d) determining the extent to which the detectable reporter gene has been activated.

4.(once amended) The method of Claim [3] 1, wherein the first or second [interacting molecule hybrid protein is [a macromolecule and may be a protein, a DNA, or a RNA] provided by introducing into the host cell a first or second chimeric gene capable of being [transcribed] expressed in the host cell.

5.(once amended) The method of Claim 4, wherein the first chimeric gene comprises a first exogenously activatable promoter, a sequence coding for a DNA binding region or polypeptide, and a sequence coding for the [first macromolecule] bait polypeptide.

8.(once amended) The method of Claim [7] 4, wherein the second chimeric gene comprises a second exogenously activatable promoter, a sequence coding for a transcriptional activation domain or polypeptide, and a sequence coding for the [second macromolecule] prey polypeptide.

9.(once amended) The method of Claim 8, wherein the second exogenously [activated] activatable promoter is activated by a second exogenous activator.

11.(once amended) The method of Claim 10, wherein at least one of the first or second exogenous activators is chosen from the group consisting of cortisol, hydrocortisone, [mineralocorticoid,] estrogen, estradiol, estrone, progesterone, androgen, ecdysone, retinoid, [steroid complementary] steroids which bind to orphan receptors, mineralocorticoid and mineralocorticoid [analogue] analogues [, or other agents capable of interacting with steroid response elements].

12.(once amended) The method of Claim 1, additionally comprising rendering the host cells capable of regulating the relative or total amounts of the first or second [interacting molecules or other macromolecules or small molecules] hybrid proteins in response to a modulatory agent acting at one or more of an extracellular, membrane, intracellular or nuclear site in order to [give at least one of a] provide continuous [or discontinuous] adjustment of a selected reporter sensitivity, wherein the modulatory agent consists of at least one of:

(a) a natural or synthetic, metabolically active or inactive steroid, steroid analogue or steroid mimic [, including glucocorticoids, dexamethasone, cortisone, cortisol, hydrocortisone, mineralocorticoids, estrogens, estradiol, estrone,

progesterones, androgens, ecdysones, retinoids, and steroids complementary to orphan receptors, mineralocorticoid or mineralocorticoid analogue, or other agent interacting with steroid responsive elements];

(b) a membrane-active agent or analogue thereof [, including an ionophore, anesthetic agent, detergent, amphoteric agent, hydrophobic agent, lipid-active agent, solvent, transmembrane signaling agent, intramembrane signaling agent, and farnesylating agent];

(c) a small molecular pharmaceutical agent [, including an antimicrobial agent, anti-tumor agent, nucleic-acid binding agent, cytoskeletal active agent, chelator, inducer, co-repressor, and agents affecting intracellular trafficking, localization, protection and degradation of exogenous or endogenous mediators, hormones and molecules];

(d) a biomolecule or natural or synthetic biopharmaceutical [, including growth factors, cytokines, hormones, and their cellular receptors and fragments and mimetics thereof].

16.(once amended) The method of Claim 12, wherein an agent capable of interfering with function of the modulatory agent is added to regulate the relative or absolute amounts of the first or second [interacting molecules] hybrid proteins.

17.(once amended) The method of claim 1, wherein the host cell is from a *Saccharomyces cerevisiae* strain [containing] comprising three integrated reporters for the detection of two-

hybrid interactions, the first integrated reporter being a construct yielding a quantifiable product, the second and third integrated reporters being [suitable, when activated, for the rescue of] constructs yielding proteins sufficient to rescue nutrient auxotrophies; wherein the first [interacting molecule] hybrid protein is provided by

(a) introducing into the host cell a plasmid containing an ampicillin or kanamycin resistance [genes] gene, a colE1 origin of replication and a DNA sequence encoding a first hybrid protein comprising a bait polypeptide and a [Gal4pBD] Gal4p DNA binding domain, the expression of which is controlled by an integrated estrogen-[inducable] inducible promoter; then

(b) inducing expression of the first hybrid protein by incubating the host cell [in] with an exogenous activator capable of activating [inducing] the promoter; and wherein

the second [interacting molecule] hybrid protein is provided by

(a) introducing into the host cell a plasmid containing an ampicillin or kanamycin resistance [genes] gene, a colE1 origin of replication and a DNA sequence encoding a second hybrid protein comprising a prey polypeptide derived from a library and the carboxyl-terminal end of the [Gal4pAD] Gal 4p transcriptional activation domain, the expression of which is controlled by a rat glucocorticoid-[inducable] inducible promoter; then

(b) inducing expression of the second hybrid protein by incubating the host cell [in] with an exogenous activator capable of activating the promoter.

Please add new Claim 18-21 as follows:

18.(new) The method of Claim 12, wherein the natural or synthetic, metabolically active or inactive steroid, steroid analogue or steroid mimic is selected from the group consisting of glucocorticoids, dexamethasone, cortisone, cortisol, hydrocortisone, estrogens, estradiol, estrone, progesterones, androgens, ecdysones, and steroids which bind to orphan receptors, and mineralocorticoid or mineralocorticoid analogues.

19.(new) The method of Claim 12, wherein the membrane-active agent or analogue thereof, is selected from the group consisting of an ionophore, an anesthetic agent, a detergent, an amphoteric agent, a hydrophobic agent, a lipid-active agent, a solvent, a transmembrane signaling agent, an intramembrane signaling agent, and a farnesylating agent.

20.(new) The method of Claim 12, wherein the small molecular pharmaceutical agent is selected from the group consisting of antimicrobial agents, anti-tumor agents, nucleic-acid binding agents, cytoskeletal active agents, chelators, inducers, co-repressors, and agents affecting intracellular trafficking, localization, protection and degradation of exogenous or endogenous mediators, hormones and molecules.

21.(new) The method of Claim 12, wherein the biomolecule or natural or synthetic biopharmaceutical is selected from the group consisting of growth factors, retinoids, cytokines, hormones, and their cellular receptors and fragments and mimetics thereof.